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ARTICLE

Genotype and cognitive phenotype of patients with tuberous sclerosis complex

Agnies M van Eeghen[1,2], Margaux E Black[1], Margaret B Pulsifer[3], David J Kwiatkowski[4] and Elizabeth A Thiele*[,1]

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disorder, which affects 1 in 6000 people. About half of these patients are affected by mental retardation, which has been associated with *TSC2* mutations, epilepsy severity and tuber burden. The bimodal intelligence distribution in TSC populations suggests the existence of subgroups with distinct pathophysiologies, which remain to be identified. Furthermore, it is unknown if heterozygous germline mutations in *TSC2* can produce the neurocognitive phenotype of TSC independent of epilepsy and tubers. Genotype–phenotype correlations may help to determine risk profiles and select patients for targeted treatments. A retrospective chart review was performed, including a large cohort of 137 TSC patients who received intelligence assessment and genetic mutation analysis. The distribution of intellectual outcomes was investigated for selected genotypes. Genotype–neurocognitive phenotype correlations were performed and associations between specific germline mutations and intellectual outcomes were compared. Results showed that *TSC1* mutations in the tuberin interaction domain were significantly associated with lower intellectual outcomes ($P<0.03$), which was also the case for *TSC2* protein-truncating and hamartin interaction domain mutations (both $P<0.05$). *TSC2* missense mutations and small in-frame deletions were significantly associated with higher IQ/DQs ($P<0.05$). Effects related to the mutation location within the *TSC2* gene were found. These findings suggest that *TSC2* protein-truncating mutations and small in-frame mutations are associated with distinctly different intelligence profiles, providing further evidence that different types and locations of *TSC* germline mutations may be associated with distinct neurocognitive phenotypes.
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INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant, multisystem disorder caused by heterozygous mutations in the tumor-suppressor genes *TSC1* and *TSC2*.[1,2] Their protein products, hamartin and tuberin respectively, interact to form a protein complex that inhibits signal transduction to the downstream effectors of the mammalian target of rapamycin complex 1 (mTORC1), a serine-threonine kinase with major roles in cell growth signaling. Various regions in the *TSC2* C-terminal domain, including the GTPase-activating protein (GAP) domain, appear to be important for normal TSC2 protein function in the mTOR pathway regulation of mTOR.[3] Mutations in *TSC1* and *TSC2* are typically inactivating, resulting in little to no protein activity, leading to upregulation of mTORC1.[4,5] This results in a constitutive growth phenotype with development of hamartomas in various organ systems, including the brain. More than 90% of individuals with TSC show neuroanatomical abnormalities such as tubers, sub-ependymal growths and white matter abnormalities.[6] Most patients are affected by epilepsy, often presenting with infantile spasms (IS) as the initial symptom of the disorder.

The prevalence of mental retardation (MR) in TSC is estimated to be between 44 and 70% and has been associated with tuber burden, tuber/brain proportion, early seizure onset, IS, mixed seizure types, *TSC2* mutations and poor seizure control.[7–13] A bimodal intellectual quotient (IQ) distribution in the total TSC population has been suggested[7] and was recently refined as being observed only in the *TSC2* population.[10]

TSC patients with germline *TSC1* and *TSC2* mutations have only one fully functional *TSC2* allele in all their cells, and this condition could lead to neurocognitive dysfunction through the mechanism of haplo-insufficiency,[14–16] similar to Fragile-X syndrome and Neurofibromatosis type 1.[14–16] However, in TSC there are additional factors which may contribute to cognitive impairment, including loss of heterozygosity, which may contribute to tuber development,[17,18] and effects of early onset and refractory epilepsy. Thus far, no associations have been found between specific TSC mutation types and cognitive outcomes,[10,19] although there are reports on associations with epilepsy and psychiatric features.[10,19–22] As most of these studies have limited power or do not address all mutation types of interest, more extensive investigations are warranted to determine potential correlations between genotype and neurocognitive phenotype in TSC. Furthermore, as mTOR-inhibitors are now under investigation to prevent or reverse neurocognitive morbidity in TSC, more specific information on genotype–phenotype associations will assist clinicians and caregivers in these important treatment decisions. In this study, we use quantitative intelligence outcomes and genetic mutation results of a large TSC patient cohort to explore the intellectual phenotype and associations with the affected gene and specific gene domains, mutation types and locations.

[1]Department of Neurology, The Carol and James Herscot Center for Tuberous Sclerosis Complex, Massachusetts General Hospital, Boston, MA, USA; [2]ENCORE, Expertise Centre for Neurodevelopmental Disorders, Department of Neuroscience, Erasmus Medical Centre, Rotterdam, The Netherlands; [3]Department of Psychiatry, Psychological Assessment Center, Massachusetts General Hospital, Boston, MA, USA; [4]Translational Medicine Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA, USA
*Correspondence: Dr EA Thiele, Department of Neurology, The Carol and James Herscot Center for Tuberous Sclerosis Complex, Massachusetts General Hospital, 175 Cambridge Street, Suite 340, Boston, MA 02114, USA. Tel: +1 617 726 0234; Fax: +1 617 726 0230; E-mail: ethiele@partners.org
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MATERIALS AND METHODS

Study group

The charts of all 377 patients with a definite diagnosis of TSC who were treated at the Herscot Center for TSC at Massachusetts General Hospital (MGH) were reviewed. TSC patients who had received genetic mutation analysis and neuropsychological assessment at the MGH Psychology Assessment Center were identified. This study was approved by the Institutional Review Board of MGH.

Cognitive assessment

Comprehensive neuropsychological evaluations, including intellectual functioning, were performed by an experienced neuropsychologist (MP). For all patients, the outcome of the most recent full-scale intelligence quotient (IQ) assessment was selected. These were available by one of the following five neuropsychological measures, according to best practice standards: (1) Bayley Scales of Infant Development – 2nd edition (BSID),[23] (2) Stanford–Binet Intelligence Scale – 5th edition,[24] (3) Wechsler Preschool and Primary Scale of Intelligence – 3rd edition,[25] (4) Wechsler Intelligence Scale for Children – 4th edition[26] and (5) Wechsler Abbreviated Scale of Intelligence – revised.[27] The BSID and Stanford–Binet also provide mental age scores, which are based on a patient's raw score converted to a mental age at which an average child would obtain that score. For the patients who were at the floor of the age-appropriate standardized scores, we calculated developmental quotients (DQs) (mental age/chronological age $\times 100$), where a DQ of 100 would be considered the mean. The presence of MR was recorded for each patient with a score of <70, according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition.

Clinical data

Clinical data were collected from the patient medical records, including information on gender, history of epilepsy and history of IS. We did not have access to clinical data of two patients, and these missing values were excluded when determining percentages in the results.

Genetic analysis

All patients at MGH followed for TSC are offered genetic testing as part of their comprehensive evaluation. Genetic testing of the *TSC1* and *TSC2* genes, including detecting of large DNA deletions and rearrangements of the *TSC2* gene, was performed at Athena Diagnostics (Worcester, MA, USA) or the MGH Neurogenetic Diagnostic Laboratory (Boston, MA, USA). Pathogenic mutations were confirmed by consultation of two TSC mutation databases (website tsc-project.partners.org, chromium.liacs.nl/LOVD2/TSC). Patients with predicted disease-associated mutations of the *TSC1* and *TSC2* genes were labeled as such. Patients with no definite findings or only polymorphisms were classified as having no mutation identified (NMI). We examined the possible effects of each of the missense mutations identified in these patients using the Alamut Mutation Interpretation Software. A single mutation (*TSC2* A460T) was predicted to have a possible effect on splicing, with a score of -33%. However, as this score was $<50\%$, it was considered unlikely to have an effect on splicing. Patients with two pathogenic mutations were excluded. An individual's specific mutation-type and its exon and nucleotide location within the *TSC1* or *TSC2* gene were recorded.

To examine the neurocognitive impact of mutations in specific gene domains, functional domains of the *TSC1* and *TSC2* gene products were selected, including the *TSC1* tuberin interaction domain (TID), the *TSC2* hamartin interaction domain (HID) and the *TSC2* GAP domain.[5,28] Mutations were additionally classified into protein-truncating (PT; nonsense, frame-shift, splice site, large deletions of at least one exon) and non-truncating (missense, small in-frame deletions and insertions) mutations. Protein-truncating mutations were divided into proximal and distal mutations, determined from the middle exon of each gene.

To investigate the effect of gene location of *TSC2* missense mutations, these were grouped into three subsets according to the exon (E) location of the affected amino acid: affecting or potentially affecting HID–TID (E1–E22); the GAP domain (E34–E41); and mutations in between these two regions (E23–E33).

Statistical analysis

Statistical analyses were performed using SPSS Version 11.5 (SPSS, Inc., Chicago, IL, USA).

T-tests were used to compare selected genetic mutation domains and types with outcomes on intellectual measures. Owing to relatively small sample size, we restricted analyses in the *TSC1* cohort to comparing TID mutations with all remaining mutations and with only proximal PT mutations.

For *TSC2* mutations, HID mutations were compared with all other mutations and proximal PT mutations ($<$E22). Additionally, GAP mutations were compared with other distal mutations, PT mutations were compared with missense mutations and small in-frame deletions, and proximal PT mutations were compared with distal PT mutations. To investigate if, within the *TSC2* cohort, PT mutations and missense mutations showed distinctly different intellectual profiles, a two-sample Mann–Whitney test was performed.

When not specifically mentioned, all mutation subgroups were compared with the remaining cohort of the respective affected gene. All reported *P*-values used two-tailed tests of significance with α set at 0.05.

RESULTS

Patient characteristics

Of all 377 patients with a definite diagnosis of TSC, 164 (44%) had received IQ/DQ assessment. Genetic testing had been performed on 137 (85%) of these patients, including 66 men and 71 women, with a mean age of 17 years old (range 3–57) and 4 patients under the age of 5. Of this study group of 137 patients, 35 (26%) patients were affected by a pathogenic *TSC1* mutation, 81 (59%) patients by a pathogenic *TSC2* mutation and in 21 (15%) patients no mutation could be identified (NMI). The distribution of the pathogenic mutations within the *TSC1* and *TSC2* genes is shown in Figure 1.

Intellectual profiles of TSC1, TSC2 and NMI cohorts

Of the total study group, the mean IQ/DQ was 71.1 (range 7–135). In 36 (22%) patients, conversion to DQ was performed. The prevalence of MR was 23% for the *TSC1* population, 57% for *TSC2* patients and 29% for the NMI cohort (Figure 1, Table 1). Intelligence scores for the total study group and according to mutation subtype are illustrated in Figure 2a and confirm the bimodal appearance of the IQ/DQ distribution of the total and *TSC2* cohort (Figures 2a and b). The mean IQ/DQ of the TSC1 and TSC2 mutation subgroups was 83 and 64, respectively, where male and female patients showed identical mean IQ/DQs (Figure 3a). The mean IQ/DQ of the total NMI subgroup was 79, with the 13 men showing a mean IQ/DQ of 77, and the 8 women a mean of 84. Of note is that the NMI subgroup consisted of 13 men and 8 women while the TSC1 and TSC2 cohorts each had a slight preponderance of women.

Genotype–phenotype analyses TSC1 mutations

In the *TSC1* cohort, the 11 (33%) patients with a mutation in the TID showed a significantly lower mean IQ/DQ of 66 ($P<0.03$) compared with 88 in the remaining *TSC1* cohort (for epilepsy characteristics, see Table 1). Compared with patients with proximal PT mutations ($<$E15) not affecting the TID domain who showed a higher mean IQ/DQ of 84, the IQ/DQs of patients with TID mutations remained lower, although not significantly ($P<0.12$). Of the four *TSC1* missense mutations, one patient had an IQ of 37, which lowered the mean of the other three related patients who had IQ/DQs between 72 and 103. Of interest are the relatively high IQ/DQs associated with the three splice site mutations affecting the proximal TID, contrary to the more distal splice site mutation in I14, which was associated with an IQ/DQ of 21. Excluding this latter splice site mutation, no *TSC1* patients with a mutation distal of the TID were affected by MR or IS, although most had a positive history of epilepsy (Figure 1 and Table 1).

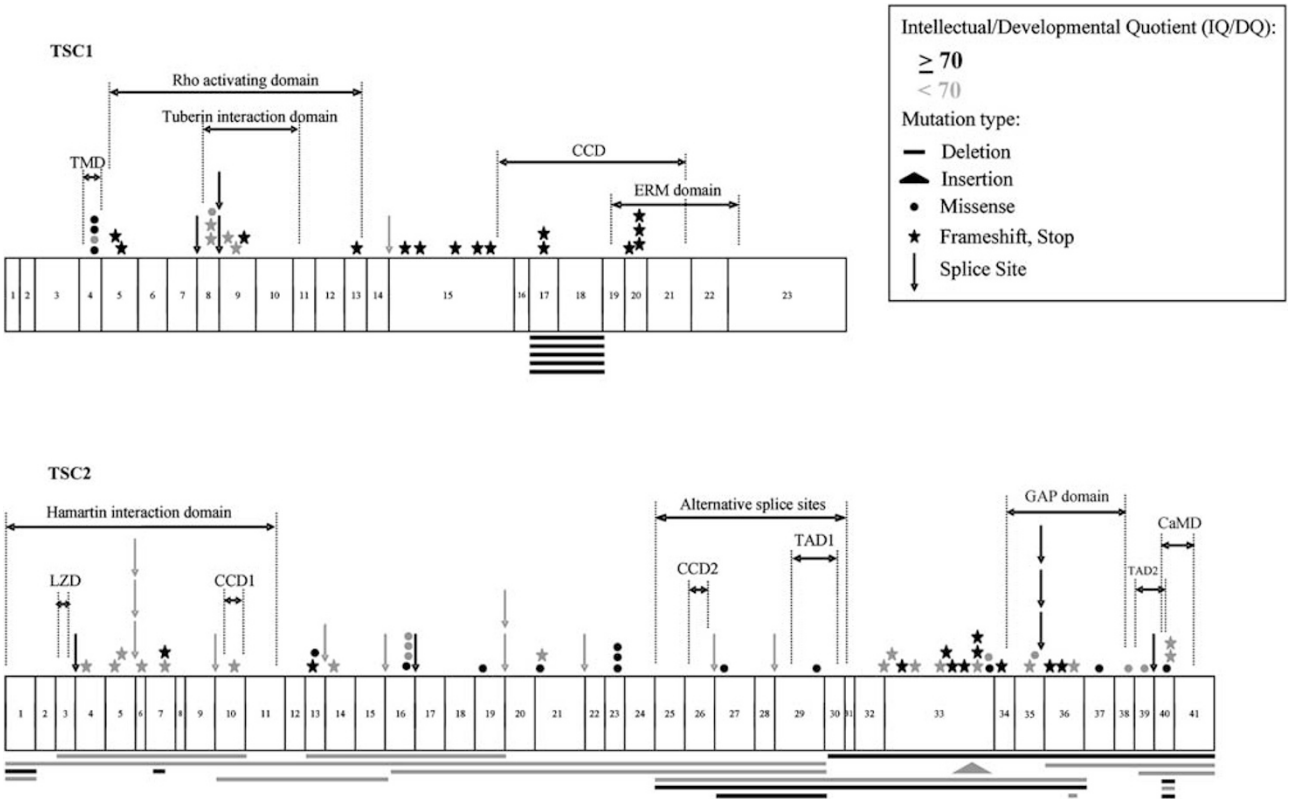

Figure 1 *TSC1* and *TSC2* gene exon map, depicting mutation types of patients with and without MR. CaMD, calmodulin-binding domain; CCD, coil–coil domain; ERM, ezrin–radixin–moesin; GAP, GTPase-activating protein; LZD, leucine zipper domain; TAD, transcription-activating domain; TMD, transmembrane domain.

Table 1 Neurocognitive characteristics per TSC mutation type and domain

Gene	*Domain/type*	*Mean IQ/DQ (range) P/N*	*MR P/N*	*Epilepsy P/N*	*IS P/N*
TSC1	All mutations	83 (7–130)	8/35 (23%)	31/35 (89%)	3/31 (1%)
	TID	66 (7–115)	5/8 (63%)	8/8 (100%)	2/8 (25%)
	Missense	72 (37–103)	2/5 (40%)	5/5 (100%)	2/5 (40%)
TSC2	All mutations	64 (7–134)	46/81 (57%)	72/81 (89%)	44/75 (59%)
	HID	51 (8–134)	14/18 (78%)	17/18 (94%)	10/16 (63%)
	GAP	72 (11–132)	5/11 (46%)	9/11 (82%)	8/10 (80%)
	PT	60 (7–134)	30/47 (65%)	42/47 (91%)	30/47 (73%)
	Prox. PT (E1-E22)	49 (22–91)	30/46 (78%)	9/9 (100%)	5/8 (63%)
	Small in-frame	79 (11–117)	8/23 (35%)	19/23 (83%)	9/22 (41%)
	Missense	78 (11–116)	6/18 (33%)	14/18 (78%)	7/17 (41%)
	Large in-frame deletions	51 (8–101)	9/13 (69%)	13/13 (100%)	6/13 (46%)
NMI	—	79 (12–135)	6/21 (29%)	13/21 (62%)	8/21 (38%)

Abbreviations: E, exon; GAP, GTPase-activating protein; HID, hamartin interaction domain; IQ/DQ, intellectual/developmental quotient; IS, infantile spasms; MR, mental retardation; NMI, no mutation identified; P/N, number of patients in cohort displaying symptoms/number of patients at risk; PT, protein truncating; TID, tuberin interaction domain; TSC, tuberous sclerosis complex.
Characteristics of the NMI cohort are included.

Genotype–phenotype analyses TSC2 mutations

Within the *TSC2* cohort, the patients with mutations in the HID (n=18, 21%) showed a significantly lower mean IQ/DQ of 51 ($P<0.05$) (for epilepsy characteristics, see Table 1). The patients with a proximal PT mutation (<E22, excluding HID mutations) showed a similar mean IQ/DQ of 49. Distal PT mutations showed a significantly higher mean IQ/DQ of 69 ($P<0.04$) compared with proximal PT mutations (Figures 2b and 3b). When PT mutations were compared with small in-frame deletions and missense mutations combined, the latter group had a significantly higher mean IQ/DQ of 76 ($P<0.05$), which was only slightly higher with an IQ/DQ of 78 when only missense mutation were included in the analysis ($P<0.04$) (Figures 2b and 3b). The Mann–Whitney test confirmed significantly different intellectual profiles for PT and missense mutations. Mutations in the GAP-domain were associated with a mean IQ/DQ of 72, which was higher than remaining mutations with a mean IQ/DQ of 63, but not significantly so ($P<0.38$). Although the GAP-related IQ/DQ profile was higher than the mean IQ/DQ of all PTs ($P<0.12$), it was only slightly higher than distal PTs (Table 1). When missense GAP mutations were compared with the remaining GAP mutations, the mean IQ/DQs were similar (71 *vs* 68).

Grouping all *TSC2* missense mutations according to their position on the gene (see Materials and Methods) revealed mean IQ/DQs that were relatively lower for proximal and distal missense mutations, whereas missense mutations in the middle of the *TSC2* gene were associated with a relatively normal cognitive phenotype, excluding one outlier (Figure 4).

DISCUSSION

The data provided by our large study cohort and quantitative intelligence outcomes are the first to indicate significant relationships between specific mutation types and intellectual outcomes in patients with TSC. We confirm the more severe neurocognitive phenotype of the total *TSC2* population, and within this cohort, found subgroups showing significantly different intellectual profiles associated with

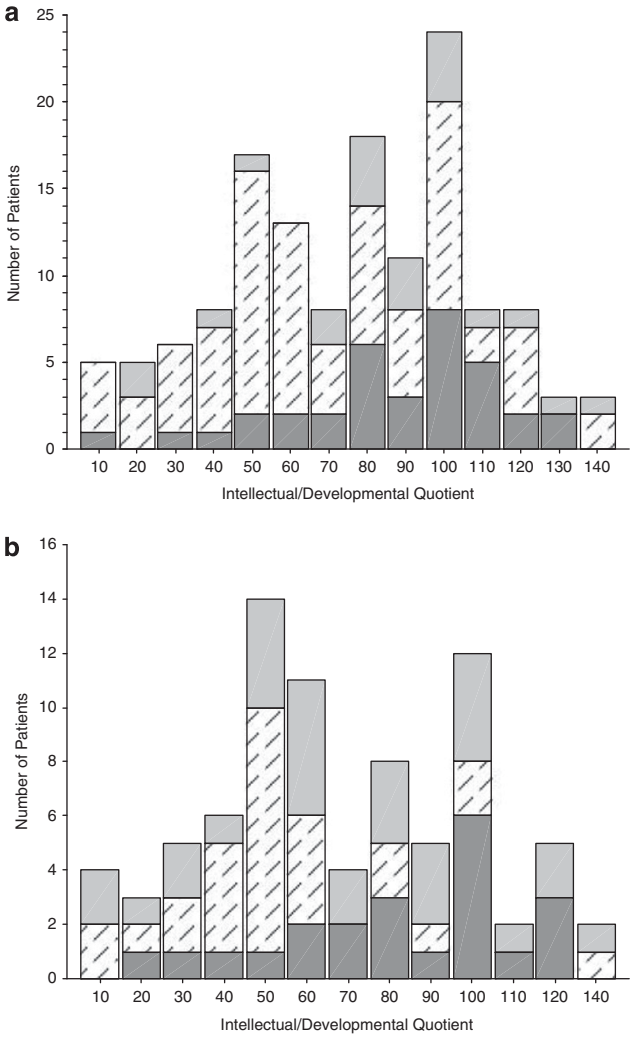

Figure 2 Histograms depicting intelligence distributions of selected *TSC* mutation groups. (**a**) Intelligence outcomes of the total TSC cohort, including the *TSC1* (dark gray), *TSC2* (shaded gray) and NMI (light gray) cohorts. (**b**) Intelligence outcomes of *TSC2* mutation subgroups with missense mutations and small in-frame deletions (dark gray), proximal protein-truncating mutations (shaded gray) and distal protein-truncating mutations (light gray).

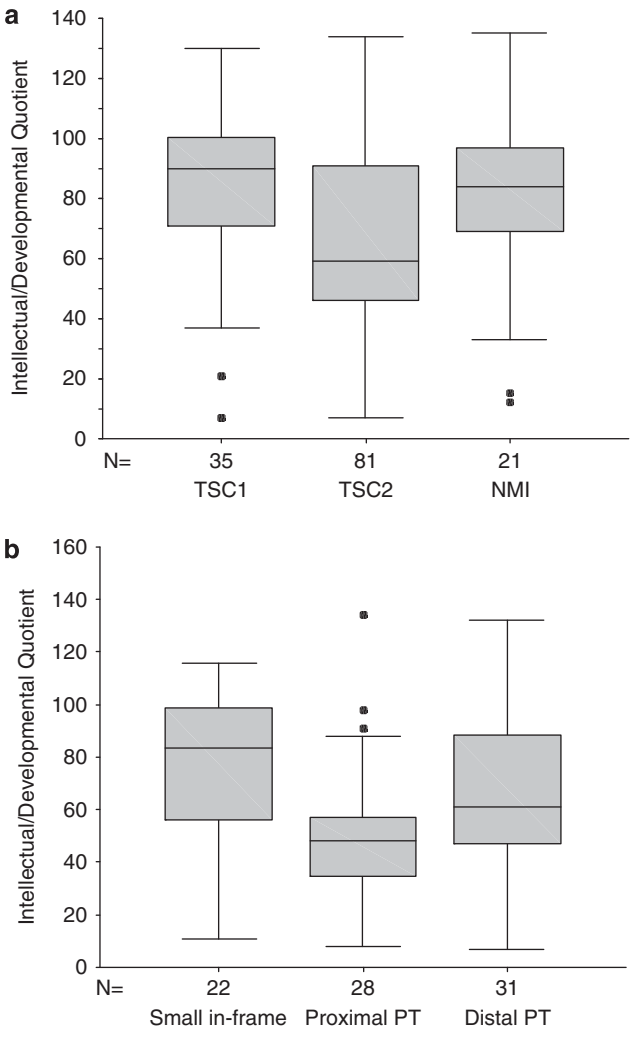

Figure 3 Boxplots depicting intelligence outcomes of selected *TSC* mutation cohorts. (**a**) Intelligence outcomes of *TSC1*, *TSC2* and NMI mutation cohort, including the mean intelligence, SD and outliers. (**b**) Intelligence distributions for *TSC2* subgroups with small in-frame deletions, proximal protein-truncating mutations, distal protein-truncating mutations. Small in-frame mutations included missense mutations and deletions <1 exon.

specific genotypes. *TSC2* patients with proximal PT mutations and HID mutations showed very similar, significantly lower mean IQ/DQs compared with patients with small, in-frame deletions or missense mutations, confirming case reports finding a milder phenotype in patients with missense mutations.[20,22,29–31] This apparent phenotypic dichotomy corresponds with the reported bimodal IQ/DQ distribution in the *TSC2* population and we showed that this bimodal appearance can, at least partly, be explained by the effects of different mutation subtypes. Furthermore, these findings correlate well with functional considerations of the effects of different mutations on the TSC1–TSC2 protein complex. Two pathophysiological mechanisms have been reported in TSC, where truncating *TSC1* and *TSC2* mutations undergo mRNA nonsense-mediated decay, and what little aberrant truncated protein is produced is likely rapidly cleared from cells in the cytosol with no functional protein production. In contrast, missense and other small in-frame mutations may produce an intact, albeit dysfunctional, protein that remains present in the cell with variable remaining function.[32,33] In addition to this 'all-or-nothing' theory, we found that PT mutations occurring in the latter half of TSC2 were associated with significantly higher intellectual outcomes than PT mutations in the first half of the protein, suggesting that mutations in *TSC2* which leave the HID intact may result in production of some functional protein. This suggests a third pathophysiological mechanism, applying to distal *TSC2* truncating mutations that leave the HID intact and result in appropriate formation of the hamartin–tuberin complex, but perhaps disrupt functions exerted by domains in the distal part of *TSC2*, such as GAP-expression, transcription and binding of kinases.[22,34] There was some suggestion that mutations in the GAP domain were associated with a relatively better neurocognitive profile, although this did not reach significance, perhaps because the relatively small sample size limited the power of this observation as we only investigated mutations directly in this domain.

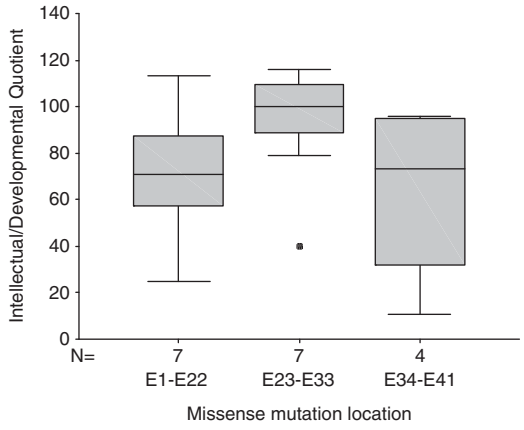

Figure 4 Boxplot depicting intelligence outcomes for all patients with *TSC2* missense mutations, specified per location of the mutation on the *TSC2* gene. The outlier in the middle group represents a female patient with a neurological history of refractory partial complex seizures and epilepsy surgery; genetic analysis revealed a P1497R mutation.

Investigating the location of mutations more in detail, we found compelling clinical support for previous observations that *TSC2* missense mutations that do not affect the hamartin–tuberin interaction or the GAP domain produce a milder phenotype, possibly by retaining some GAP activity.[31,35] The single severely affected patient in the 'milder' missense group possibly reflects a remote splice site mutation,[36] a severe 'second hit' in the other allele, secondary effect of seizures or another pathophysiologic phenomenon in this complex syndrome.

Although the *TSC1* cohort was relatively smaller, the findings that mutations in the TID were associated with a more severe cognitive phenotype, even when compared with only proximal PT mutations, confirms the importance of unimpaired binding of hamartin and tuberin through their interaction domains. The three patients with splice site mutations in the TID without cognitive impairment were noteworthy and future studies should focus on protein studies of splice site mutations to learn more about their effect. The relatively low mean IQ/DQ of the five *TSC1* missense mutations conflicts with previous observations of a relatively mild phenotype,[33,37] possibly due to the fact that all of these missense mutations occurred in the N-terminal region, which is essential for TSC1 function.[32,38] Of note is that, excluding splice site mutations, none of the patients with more distal mutations were affected by MR.

Previous studies have reported a more severe cognitive phenotype in men compared with women with TSC, using dichotomous outcomes such as 'MR'.[19,39,40] However, the nearly identical IQ/DQs in men and women in our large *TSC1* and *TSC2* cohorts are more consistent with previous data on the prevalence of autism, ADHD and other neuropsychiatric disorders in the TSC population,[41,42] suggesting that genetic effects override gender effects.

The neurocognitive phenotype of patients with TSC is highly variable, because of several effects. Apart from the effect of the genetic mutations, the effect of epilepsy comorbidity, neurosurgery and other anti-epileptic treatments may influence cognitive development in patients with TSC and thus complicate genotype–phenotype associations. Our explorations confirm that cognition and epilepsy are interrelated in TSC, showing greater frequencies of epilepsy and IS in mutation subgroups with a higher prevalence of MR and low mean IQ/DQs, which may contribute to the large ranges of IQ/DQ per mutation subgroup. In addition, it is still unclear if second hits are absolutely necessary for the formation of tubers,[17,18] which are also associated with intellectual outcomes.[9,43]

A drawback for this type of study is the use of multiple cognitive measures, which is inherent to the inclusion of different age groups. We accounted for this by using both dichotomous and quantitative outcomes of cognitive functioning, MR and IE, in order to validate and strengthen our findings. As the patients were assessed at different ages, it is unclear if cognitive development is sufficiently stable in patients with TSC to perform such a study. Although thus far there has been little investigation on cognitive development in children and adults with TSC, we recently found that the mean IQ/DQ of a large TSC cohort remained stable over time, albeit showing variability,[44] confirming a previous study in infants with TSC.[45] As this study group represents only TSC patients who were referred for neuropsychological assessment, this may represent a bias toward more severely affected patients. For this study, we selected intelligence as the primary outcome because these quantitative data provide more precise and powerful information, although this reduced the size of the study cohort. We limited our statistical analysis to intelligence outcomes per mutation type, as similar investigations on epilepsy parameters are ongoing in a larger sample. Of importance is that some subjects in categories associated with a more severe neurocognitive phenotype had excellent intelligence outcomes. This, together with the described missense mutation finding, limits the use of our findings as prognostic indicators and should remind clinicians to be very cautious in attempting phenotype predictions. Future genotype–phenotype correlations in larger cohorts should expand on our findings and include seizure variables, psychiatric burden and the neuroanatomical endophenotype of TSC. Functional analysis on the biochemical effects of specific missense mutations, small in-frame deletions and splice site mutations may identify more genotype–phenotype correlations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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